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EVALUATION OF THE PARIETAL BONES IN THE RAT AS A SPECIFIC SITE FOR THE TESTING OF OSTEOGENIC MATERIALS

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SHORT TITLE: A Simple Animal Model to Study Bone Implant Material

*Based on a section of the thesis to be submitted to the Graduate Faculty, University of Maryland, in partial fulfillment of the requirements of the Ph.D. degree.

SYNOPSIS

A full-thickness periosteal scalp flap overlying the parietal bones of rats was made and a trephine was used to prepare 2.5 mm diameter osseous defects to the pia mater. Healing was evaluated by gross and histologic examination at two-week invervals for eight weeks using a Zeiss Videoplan Image Analysis System with Osteoplan.™ None of the osseous defects healed by bony union.

INTRODUCTION

Many substances have been investigated to determine if they possess osteogenic potential.[1-5] The specific site in the experimental animal where an osteogenic implant material will be evaluated will have a profound influence on the observed results.

Investigators have prepared osseous defects in monkeys, sheep, and dogs in an attempt to study the effects of bone inducing agents. These animals offer excellent tissue sites where bony healing can be investigated. Unfortunately, large animals are expensive to purchase, costly to feed and maintain, and they require significant housing space. Small animals such as rodents and rabbits are an expedient alternative. The mandible, major trochanter, and the metaphysis and diaphysis of endochondral bones of small animals have often been selected as experimental wound sites. However, bony cavities prepared in these areas in control animals frequently displayed osseous regeneration without any implant or graft. Melcher and Irving[6] and Pallasch[7] stated that spontaneous bone production will occur in defects less than 5 mm in diameter. If this is the case, then evaluating a material for its osteogenic potential in such a site is unsatisfactory.

Spontaneous repair of experimental wounds in intramembranous bone is poor.[8-10] Purportedly, osseous defects in this type of bone do not heal by bony ingrowth; but rather, by the production of fibrous tissue. The purpose of this study, therefore, was to determine if a 2.5 mm diameter osseous defect prepared in the parietal bones of rats would heal by bony growth.

MATERIALS AND METHODS

Twenty adult Walter Reed strain albino rats, weighing approximately 300 to 325 gms each were anesthetized with sodium pentobarbital (6mg/kg) intraperitoneal. The heads and necks were shaved and scrubbed with povidone iodine N.F. (Betadine®*) for three to five minutes and rinsed with sterile water. A midsagittal incision was made through the skin of the scalp, and flaps were reflected to expose soft tissue covering the calvarium (specifically, parietal bones). The fascia overlying the bones was dissected away, exposing periosteum. A trephine with an O.D. of 2.5 mm and sterile water coolant were used to prepare a 2.5 mm hole through periosteum and the right and left parietal bones, exposing dura mater. The surgical sites were irrigated with sterile saline to remove bony debris. The skin flaps were then reapposed and sutured.

Five animals were sacrificed at two-week intervals for eight weeks with an overdose of sodium pentobarbital. After the animals were sacrificed, skin flaps were reflected from the experimental area of the calvarium. Using a pencil-diamond bur in a water cooled air rotor, the calvarium was sectioned so that the right and left parietal defects could be recovered. At least 3 to 5 mm of bone surrounded each retrieved defect. The tissue specimens were fixed in buffered formalin and next decalcified for 18 hours in Bankothy's medium. The prepared specimens were sectioned at 6 microns and stained with hematoxylin and eosin for histomorphometric evaluation using a Zeiss Videoplan Image Analysis System with Osteoplan.™

*Betadine®, Purdue Frederick Company, Norwalk, Connecticut

RESULTS

Gross Examination

None of the prepared parietal bone defects displayed any evidence of osseous healing. As early as two weeks a complete fibrous bridge could be observed covering the wound sites. The pattern of fibrous tissue closure was also clearly evident at four to eight weeks.

Histologic Examination

The appearance of the specimens histologically confirmed the gross examination. Distinct wound edges were bordered by bone (Figure 1). Dense, irregular collagenous connective tissue of the defect contrasted dramatically with the organized nature of the parietal bones (Figure 2). The histologic picture remained unchanged throughout the eight week experiment: dense, irregular swirls of collagen, eosinophyllic matrix, and occasional capillary buds (Figure 3). Osteoblasts, osteoid, and trabeculae were absent.

The Zeiss Videoplan Image Analysis System with Osteoplan™ provided compelling statistical data for a lack of hard tissue wound healing over the eight week experiment. Employing the Osteoplan's 36 parameters for histomorphometric bone evaluation, it was unequivocally determined that bone union in the parietal bone defect was completely lacking.

DISCUSSION

Several criteria should be fulfilled by both the experimental animal and the implant site that will be used for the evaluation of a material that is possibly osteogenic. Some of these important qualities are:

1. The animal should be inexpensive, readily available, and easily handled and anesthetized.

2. The animal should have an adequate amount of bone at the test site so that spontaneous healing will not occur in the control animals. Sufficient bone must also be available so that the risk of fracture is mitigated.

3. The animal should allow for accurate follow-up and assessment of the implant and of the contiguous host tissue.

4. The implant site should consist of both cortical and cancellous bone.

5. The procedure for preparing the implant area should be simple, rapid, and result in low or no animal morbidity.

6. The procedure should result in a predictable, consistent response from the implant area.

An animal model system consisting of the parietal bones of the rat appropriately satisfies all necessary criteria and also provides a highly satisfactory means for evaluating the osteogenic potential of bone implant agents. Bony cavities prepared in the parietal bone of the rat do not heal spontaneously; therefore, evidence of osteogenesis in a defect that was induced by an implant material would strongly implicate material as being osteogenic.

In 1946 Pritchard[11] prepared linear fractures in the parietal bones of rats. He determined that fibrous union was the usual outcome; however, osseous bridging resulted when fracture gaps were exceedingly narrow and where fragments of bone acted as grafts. Melcher[12] has suggested that parietal bone might produce a substance that inhibits the proliferation and differentiation of osteoblast precursor cells.

Consequently, defects in the parietal areas do not heal by bony union, but rather by a fibrous bridging.

Frame[13] suggested using a 15 mm defect in the cranial vault of a cross-breed of New Zealand red and half lop rabbit. He felt that this was a prudent site for testing bone substitute materials. However, the rabbit is more expensive to purchase and maintain than is the rat. In addition, the amount of implant material that may be required to fill multiple, 15 mm cavities may be both feasibly impossible to synthesize and prohibitively costly. Removal of the implant substance from the host area for histologic examination and biochemical assaying could be an extremely difficult manipulative task. The mandible has been frequently mentioned as a site for evaluating the osseous potential of implants.[13-15] However, the mandible is a unique bone because it is subjected to continuous motion and both compressive and shearing forces. Therefore, responses elicited from a mandibular host may not be typical or indicative of other host sites. In addition, prepared mandibular bone cavities often heal spontaneously.[13,15] The rat parietal bone model is an exceptional alternative to the unweildy rabbit system and the unpredictable mandibular region.

CONCLUSION

A 2.5 mm defect prepared in the parietal bones of the rat showed no evidence of bony healing after eight weeks. It may, therefore, be stated that the parietal bone of the rat can provide a legitimately acceptable and response-predictable system for evaluating bone inducing implant agents. The method for producing the bone defect is simple

and rapid. There was no animal morbidity. The rat is inexpensive, readily accessible, and specimens are easily retrieved and manipulated for both histomorphometric analyses and biochemical assays.

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

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LEGENDS

FIGURE 1. (16X) - At four weeks there was well organized connective tissue filling the prepared bony defect. Bone can be seen in top left corner.

FIGURE 2. (40X) - Dense, irregular connective tissue can be seen (left) in contrast to the parietal bone (right).

FIGURE 3. (16X) - Several capillary buds may be seen among the dense connective tissue that filled the parietal bone cavities.

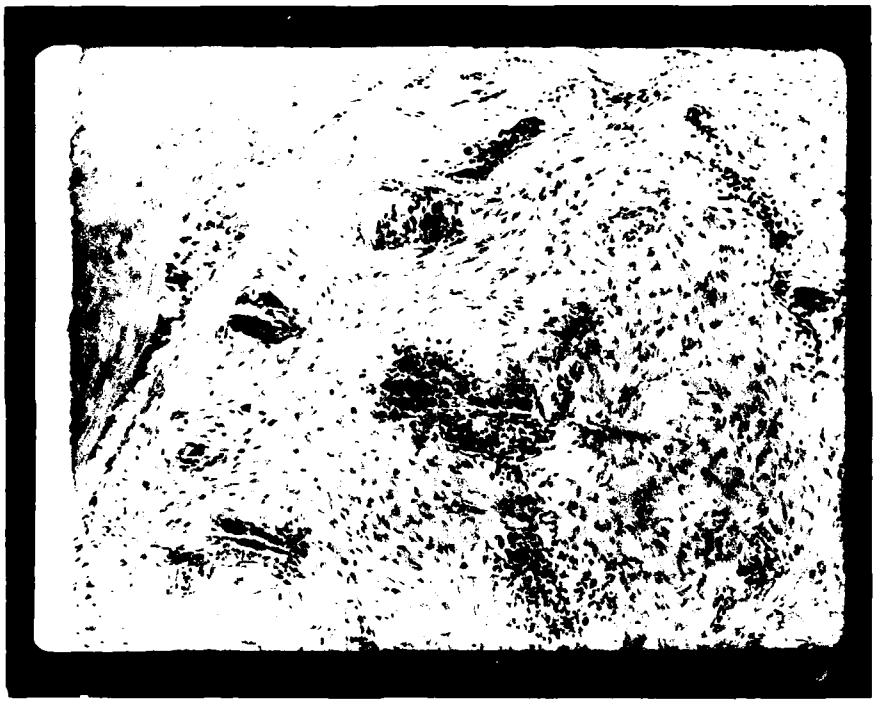

FIGURE 1

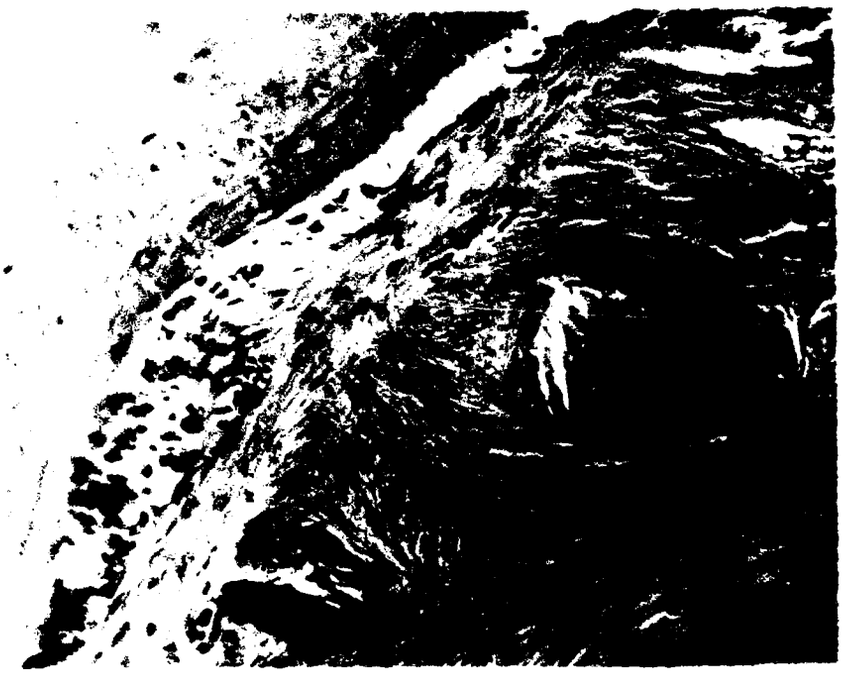

FIGURE 2

FIGURE 3


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